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Original Description of the Brown Frog from Hokkaido, Japan (Genus *Rana*)

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Abstract: ***Rana pirica*, a brown frog from Hokkaido, Japan, is described as a new species. The species is closely related to *R. ornativentris*, *R. dybowskii* and *R. chensinensis*, characterized by 2n = 24 chromosomes, but is distinct morphologically from all three, and genetically from the first two species. Systematic problems of brown frogs from east Asia are discussed.**

Key words: Ranidae; *Rana*; Systematics; Hokkaido; Chromosome number

Unlike the majority of the genus *Rana* with 2n = 26 chromosomes, some members of the brown frogs are unique in having diploid chromosomes of 2n = 24. These include the European *R. arvalis* and several east Asian forms allied to *R. chensinensis*, such as *R. ornativentris* and *R. dybowskii* (Witschi et al., 1958; Kobayashi, 1962; Seto, 1965; Green, 1983; Wu, 1982; Luo and Li, 1985; Ma, 1987; Wei et al., 1990). These frogs are quite similar in morphology, and are nostoriously difficult to classify (Nakamura and Uéno, 1963). Among them is the brown frog from Hokkaido, Japan. The population was long considered to be conspecific with the European *R. temporaria* (Boulenger, 1886; Stejneger, 1907; Okada, 1930), but, after its unique karyology was made clear, it is often referred to as *R. chensinensis* (Kawamura, 1962). The latter species, however, was originally described from western China, far distant from Hokkaido. Between these localities, there occurs another relative, *R. dybowsikii*, and studies from hybridization experiments (Kawamura et al., 1981) revealed that there is a post mating isolation mechanism between *R. dybowskii* from Tsushima or Korea and the brown frog from Hokkaido (treated as *R. chensinensis*). The disjunct distribution of conspecific populations of *R. chensinensis* both in Hokkaido and western China seems biogeographically unnatural, unless a very special case, such as a relict distribution, is taken into consideration.

Assignment of the brown frog from Hokkaido to *R. chensinensis* was made by Kawamura (1962) on the basis of literature, and one of the chief characters he stressed in this classification was the property of the oviduct that swells with water during preservation. Kawamura (1962:185) stated that this feature is seen both in the brown frog from Hokkaido and *R. chensinensis*. Actually, however, only limited populations of *R. chensinensis* from northeastern China show this characteristic (Liu and Hu, 1961:187). Although the original description of *R. chensinensis* (David, 1875:159) is brief and uniformative, and nothing is reported for the topotypic population, frogs from Beijing and Qingdao, which are morphologically so close to the topotypic population as to be considered to form together with it one distinct subspecies (Hu et al., 1966:60), are reported to lack such a unique oviducal property (Liu and Hu, 1961:187). Thus, as noted by Liu and Hu (1961:187) and Hu et al. (1966), *R. chensinensis*, the so-called Chinese brown frog is itself highly polymorphic and assuredly includes several distinct forms. From the available morphological information about the nominate population of *R. chensinensis* (Hu et al., 1966), the species is not regarded as conspecific with the brown frog from Hokkaido.

As is evident from the above brief review, the brown frog occurring in Hokkaido assuredly represents an undescribed form, and I here describe this frog as a new species, based on my own results of morphometric and electrophoretic studies.

MATERIALS AND METHODS

Morphometry.–I examined a total of 62 preserved specimens of brown frogs: *Rana* sp. from Hokkaido (15 males and 11 females), *R. ornativentris* (10 males and 10 females), and *R. dybowskii* (9 males and 7 females), all from Japan (Fig. 1; Appendix I). In order to assess morphometric similarities and differences among

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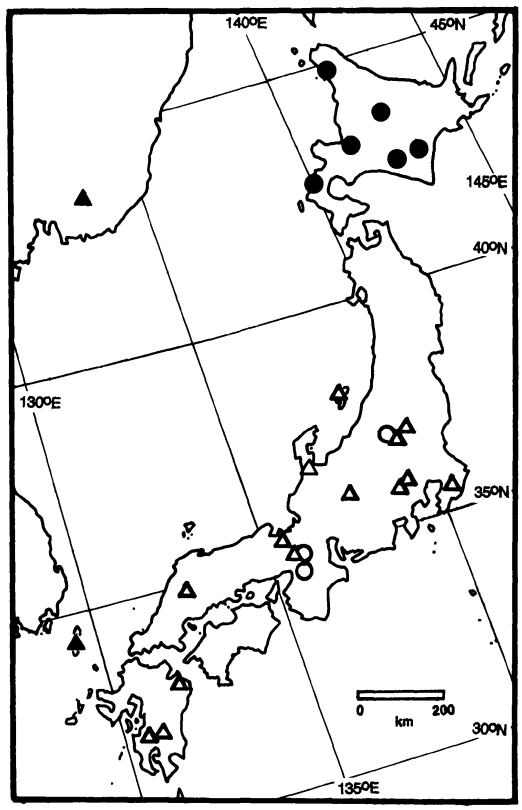

FIG. 1. Map of Japan and the Far East USSR, showing sampling localities of brown frogs used in the morphometric and electrophoretic analyses. Closed circles=*Rana* sp. from Hokkaido; Open triangles=*R. ornativentris*; Closed triangles=*R. dybowskii*; Open circles=*R. japonica*.

the samples, 15 body measurements were taken mostly following Matsui (1984). Briefly, they are: 1) snout-vent length (SVL); 2) head length (HL); 3) snout length (SL); 4) eye length (EL: length of eyeball); 5) tympanum diameter (TD); 6) head width (HW); 7) internarial distance (IND); 8) interorbital distance (IOD); 9) upper eyelid width (UEW); 10) lower arm length (LAL); 11) hindlimb length (HLL); 12) thigh length (THIGH); 13) tibia length (TL); 14) foot length (FL); and 15) inner metatarsal tubercle length (IMTL). All measurements were made to the nearest 0.1 mm with dial calipers. Only sexually matured adults were measured, and males and females were analyzed separately.

Programs designed and implemented by the SAS (1985) package were used in the statistical analysis. The analyses were performed through the facilities of the Data Processing Center, Kyoto University. A canonical discriminant analysis, performed by the CANDISC program (SAS, 1985) was conducted and canonical variate scores of individuals were plotted on their respective axes.

Electrophoresis.—For an electrophoretic examination, a total of 46 specimens belonging to six samples were employed. These include two samples of *Rana* sp. from Hokkaido (N = 9 from Sapporo and N = 9 from Obihiro), one sample of *R. ornativentris* (N = 9 from Honshu and Kyushu), two samples of *R. dybowskii* (N = 7 from Tsushima and N = 3 from Maritime, Far East USSR), and one sample of *R. japonica* (N = 8 from Honshu; Appendix II). We used *R. japonica* as an outgroup taxon for phylogenetic analyses based on morphological, ecological, and karyotypic evidence (Maeda and Matsui, 1989).

Livers were removed from freshly killed animals and frozen (−84 C) before use. Voucher specimens were fixed in 10 % formalin, later preserved in 70 % ethanol and stored in the collection of Human and Environmental Studies, Kyoto University [KUHE, formerly Matsui's collection in the Biological Laboratory, Kyoto University (MC)]. Horizontal starch gel electrophoresis was employed to resolve allozyme products (Selander et al., 1971) using 11.5% potato starch (Connaught). The allozymes examined and locus designations are listed in Table 1. Genetic interpretations of allozyme data were based on criteria developed by Selander et al. (1971). Enzyme nomenclature and E. C. numbers followed the recommendations of the Nomenclature Committee of the International Union of Biochemistry (IUBNC, 1984), and the notations of loci, electromorphs and genotypes followed principally Murphy and Crabtree (1985). Electromorphs were designated by letters with "a" representing the most rapidly migrating anodal variant.

Standard estimates of genetic variability, i. e., mean heterozygosity by direct count (H), proportion of loci polymorphic (P), and the mean number of electromorphs per locus (A), were computed for each sample.

Overall genetic differentiation among samples was estimated using coefficients of Nei's (1978) unbiassed genetic distance and modified Rogers' genetic distance (Wright, 1978). Genetic relationships among samples were estimated from the pairwise matrix of Nei's distance, clustered according to the UPGMA algorithm (Sneath and Sokal, 1973). This method assumes equal rates of molecular evolution along all branches, but

TABLE 1. Enzymes, loci, and buffer systems used in the analysis of relationships among Japanese brown frogs. Mitochondrial and supernatant loci are denoted by m and s prefixes, respectively.

Enzyme	Enzyme commission number	Locus	Buffer condition*
Aconitate hydratase	4.2.1.3	mAcon-A	C
	4.2.1.3	sAcon-A	C
Aspartate aminotransferase	2.6.1.1	mAat-A	A
	2.6.1.1	sAat-A	A
Dipeptidase	3.4.13.11	Pep-B	D
	3.4.13.11	Pep-C	D
Esterase (Umb.)	—	Est-1	A
Fructose-bisphosphatase	3.1.3.11	Fbp-A	D
Fructose-bisphosphate aldolase	4.1.2.13	Fba-A	A
Glucose dehydrogenase	1.1.1.47	Gcdh-A	C
Glucose phosphate isomerase	5.3.1.9	Gpi-A	B
Glutamate dehydrogenase	1.4.1.3	Gtdh-A	C
Glycerol-3-phosphate dehydrogenase	1.1.1.8	G3pdh-A	C
3-Hydroxybutyrate dehydrogenase	1.1.1.30	Hbdh-B	B
L-iditol dehydrogenase	1.1.1.14	Iddh-A	A
Isocitrate dehydrogenase	1.1.1.42	sIcdh-A	A
Lactate dehydrogenase	1.1.1.27	Ldh-A	A
	1.1.1.27	Ldh-B	A
Malate dehydrogenase	1.1.1.37	mMdh-A	A
	1.1.1.37	sMdh-A	A
"Malic Enzyme"**	1.1.1.40	mMdhp-A	A
	1.1.1.40	sMdhp-A	A
Phosphoglucomutase	5.4.2.2	Pgm-A	A
	5.4.2.2	Pgm-C	A
Phosphogluconate dehydrogenase	1.1.1.44	Pgdh-1	A
Superoxide dismutase	1.15.1.1	sSod-1	D
Xanthine oxidase	1.2.3.2	Xo-A	C

* Buffer systems: A=Tris-citrate, pH 7.0. B=Tris-citrate, pH 6.0. C=Tris-citrate, pH 8.0. D=Tris-borate-EDTA, pH 8.0.
** NADP-dependent malate dehydrogenase.

the validity of this assumption is not yet proven. We adopt this method to describe amounts of genetic divergence in these species and to allow comparison to literature accounts of variability in other anuran species. Alternatively, a Distance-Wagner tree (Farris, 1972) was constructed using BIOSYS-1 optimized with the multiple addition criterion of Swofford and Selander (1981) with metric measures of modified Rogers' genetic distance coefficients, and rooted by *R. japonica*.

Cladistic analyses were accomplished by either considering alleles as characters and their presence or absence as the character states (independent allele model of Mickevich and Mitter, 1981), or treating loci as characters and their allelic composition as the states (qualitative coding of Buth, 1984). The resulting character matrices were analyzed using version 3.0 of PAUP (Swofford, 1990). The branch-and-bound algorithm was used to find the shortest trees.

RESULTS

Morphometry.—Results from the CANDISC of adult females and males are presented in Fig. 2A and B, respectively. In females, the eigenvalue of the two axes accounted for 57.56 and 1.98, and the first canonical variable accounted for 96.7% of the total amount of eigenvalues. In males, the eigenvalue of the two axes accounted for 8.14 and 1.54, respectively. The first canonical variable accounted for 84.1% of the total amount of eigenvalues. On the first two axes, the separation of *Rana* sp. from Hokkaido from the other two species was complete in both sexes. The male *R. dybowskii* slightly overlapped male *R. ornativentris*, but separation was complete in females of these two species. On the first and the third axes, separation of *R.* sp. from Hokkaido from *R. ornativentris* or *R. dybowskii* was again complete in both sexes, but the latter

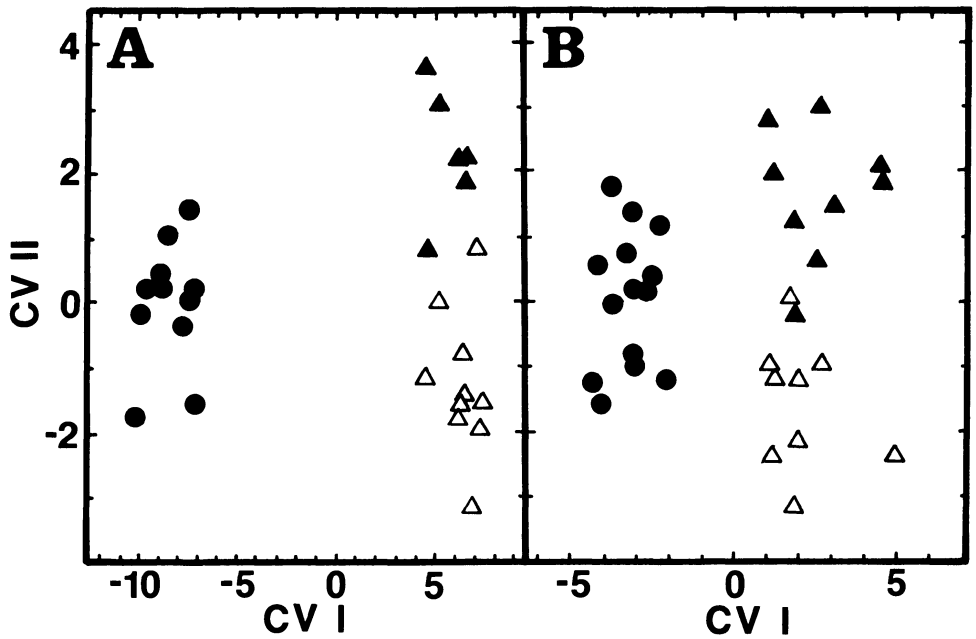

FIG. 2. Plot of first against second canonical variates for samples of *Rana* sp. from Hokkaido (closed circles), *R. ornativentris* (open triangles), and *R. dybowskii* (closed triangles). A : adult females; B : adult males.

two species showed overlap. The absolute magnitudes of the standardized canonical discriminant coefficients are proportional to the relative importance of each character in separating the three species along each canonical discriminant axis. In females, THIGH was the greatest contributor to separation on the first axis (28.976), followed by HLL (-23.724) and SVL (-4.342). On the other hand, variables with the highest contribution on the first axis in males were THIGH (4.128), HL (2.483) and HLL (-2.034). Thus, *Rana* sp. from Hokkaido is morphometrically completely distinguishable from *R. ornativentris* or *R. dybowskii* in both sexes.

Electrophoresis.–Of the 27 presumptive loci we studied, three were monomorphic (Table 2). These included mAcon-A, Gtdh-A and Ldh-A. Of the remaining 24 polymorphic loci, Hbdh-B and Pgdh-1 were most variable, each with six alleles. In all polymorphic loci, but one, the predominent electromorph occurring at frequencies of 50% or more, was identical in the two samples of *R.* sp. from Hokkaido.

In the six samples, the percentage of polymorphic loci (P) varied from 48.1–70.4, and the mean heterozygosity (H) values from 0.069–0.118 (Table 2). The highest polymorphism was found in *R. ornativentris*, which includes specimens from a wide region of Japan, while the highest heterozygosity was found in the Obihiro sample of *R.* sp. from Hokkaido.

Although the branching order differed according to the analysis used, both the phenetic and phylogenetic analyses agreed in clustering the six forms into similar units, and all agreed in placing the two samples of *Rana* sp. from Hokkaido as sister groups. The two samples of *R. dybowskii* were also clustered together in all trees, except for the UPGMA tree.

In the UPGMA tree, constructed on the basis of Nei's (1978) genetic distance (Table 3), the first major dichotomy separated *R. japonica* from all the other samples and supported the choice of making this species an outgroup taxon in the phylogenetic analyses. In the second major dichotomy, *R. dybowskii* from Tsushima was separated from the remaining samples. Whereas *R. dybowskii* from the USSR was placed as the next most similar taxon to the two Hokkaido samples, which together were separated from *R. ornativentris*.

A Distance-Wagner tree constructed using metric measures of modified Rogers' D and rooted by *R. japonica* is shown in Fig. 3. The first dichotomy separated *R. ornativentris* from the remaining samples. No marked asymmetry in branch lengths was observed in the Wagner tree. After optimization of branch lengths, the

TABLE 2. Allele frequencies and genetic variability (mean±1SE) at 27 loci in the six samples of brown frogs. A=mean number of alleles per locus; P=percentage of loci polymorphic; H=mean heterozygosity (direct count).

Locus	*Rana* sp. Sapporo (N=9)	*Rana* sp. Obihiro (N=9)	*R. ornativentris* (N=9)	*R. dybowskii* Tsushima (N=7)	*R. dybowskii* Ussuri (N=3)	*R. japonica* (N=8)
mAcon-A	a 1.000	a 1.000	a 1.000	a 1.000	a 1.000	a 1.000
sAcon-A	a 0.167	a 0.222	a 0.333	a 0.143	b 1.000	a 0.333
	b 0.833	b 0.778	b 0.667	b 0.857		b 0.667
mAat-A	c 0.778	c 0.444	c 0.556	c 0.286	b 0.333	a 0.375
	d 0.111	d 0.444	d 0.444	d 0.714	d 0.667	d 0.625
	e 0.111	d 0.111				
sAat-A	b 0.500	a 0.056	c 1.000	b 0.643	e 1.000	b 0.250
	c 0.500	b 0.500		c 0.357		c 0.375
		c 0.444				d 0.313
						e 0.063
Pep-B	a 0.813	a 0.833	a 0.938	a 0.143	a 1.000	a 0.143
	c 0.188	c 0.167	b 0.063	b 0.857		b 0.714
						c 0.143
Pep-C	a 0.167	a 0.167	b 0.111	d 0.286	d 0.500	c 0.750
	b 0.500	b 0.556	c 0.167	e 0.714	e 0.500	d 0.250
	c 0.111	c 0.056	d 0.333			
	d 0.222	d 0.222	e 0.389			
Est-1	c 1.000	c 1.000	a 0.563	a 0.500	a 0.167	a 0.125
			b 0.188	b 0.500	b 0.833	b 0.875
			c 0.250			
Fbp-A	b 1.000	b 1.000	a 1.000	a 0.143	b 1.000	c 1.000
				b 0.857		
Fba-A	a 1.000	a 1.000	a 0.056	a 0.143	a 0.833	d 1.000
			b 0.944	c 0.857	c 0.167	
Gcdh-A	b 0.500	b 0.071	c 1.000	a 0.500	c 1.000	b 1.000
	c 0.500	c 0.929		c 0.500		
Gpi-A	a 0.167	a 0.111	a 0.150	c 0.857	b 0.667	d 0.125
	b 0.167	d 0.889	b 0.400	d 0.143	d 0.333	e 0.375
	d 0.667		c 0.050			f 0.500
			d 0.400			
Gtdh-A	a 1.000	a 1.000	a 1.000	a 1.000	a 1.000	a 1.000
G3pdh-A	c 1.000	c 1.000	c 1.000	a 0.286	b 0.167	a 0.188
				b 0.500	c 0.833	c 0.813
				c 0.214		
Hbdh-B	a 0.111	a 0.125	a 0.188	d 1.000	b 0.333	d 1.000
	c 0.056	d 0.813	b 0.250		c 0.333	
	d 0.611	f 0.063	c 0.313		d 0.333	
	e 0.111		d 0.250			
	f 0.111					
Iddh-A	b 1.000	b 0.611	b 0.833	a 1.000	a 1.000	b 0.071
		c 0.389	c 0.167			c 0.929
sIcdh-A	b 1.000	b 1.000	a 0.444	a 0.286	c 1.000	d 1.000
			b 0.556	b 0.571		
				c 0.143		
Ldh-A	a 1.000	a 1.000	a 1.000	a 1.000	a 1.000	a 1.000
Ldh-B	e 1.000	e 1.000	c 0.063	a 1.000	b 0.167	d 0.875
			d 0.813		c 0.167	e 0.125
			e 0.125		e 0.667	
mMdh-A	b 1.000	b 1.000	d 1.000	c 1.000	b 1.000	a 0.375
						e 0.625

TABLE. 2. Continued.

Locus	*Rana* sp. Sapporo (N=9)	*Rana* sp. Obihiro (N=9)	*R. ornativentris* (N=9)	*R. dybowskii* Tsusima (N=7)	*R. dybowskii* Ussuri (N=3)	*R. japonica* (N=8)
sMdh-A	c 1.000	c 1.000	a 0.944	d 1.000	c 1.000	c 0.063
			b 0.056			e 0.938
mMdhp-A	a 0.889	a 0.667	a 0.389	b 0.214	c 0.667	a 0.500
	b 0.111	b 0.167	b 0.333	c 0.714	d 0.333	b 0.500
		d 0.167	d 0.278	d 0.071		
sMdhp-A	a 0.889	a 0.944	a 0.944	b 0.143	a 0.833	c 0.875
	b 0.111	b 0.056	b 0.056	c 0.857	b 0.167	d 0.125
Pgm-A	b 0.889	b 1.000	a 0.056	a 1.000	a 0.833	b 1.000
	c 0.111		b 0.944		b 0.167	
Pgm-C	a 0.278	b 1.000	a 0.444	a 0.857	b 1.000	c 1.000
	b 0.722		b 0.556	b 0.143		
Pgdh-1	e 1.000	e 0.833	d 0.056	a 0.071	a 0.667	c 0.875
		f 0.167	e 0.611	b 0.929	b 0.333	d 0.125
			f 0.333			
sSod-1	a 0.611	a 0.722	a 0.889	a 1.000	a 1.000	b 1.000
	b 0.389	b 0.278	b 0.111			
Xo-A	a 0.571	a 0.556	b 0.833	b 1.000	b 0.667	b 0.250
	b 0.429	b 0.444	c 0.167		c 0.333	c 0.750
A	1.8±0.2	1.7±0.2	2.1±0.2	1.7±0.1	1.6±0.1	1.7±0.1
P	51.9	51.9	70.4	59.3	48.1	59.3
H	0.088± 0.028	0.118± 0.030	0.105± 0.028	0.069± 0.028	0.111± 0.036	0.076± 0.023

TABLE 3. Nei's (1978) unbiased genetic distance (above diagonal) and modified Rogers' (Wright, 1978) genetic distance (below diagonal) among *Rana* sp. from Hokkaido (Samples 1, 2), *R. ornativentris* (Sample 3), *R. dybowskii* (Samples 4, 5), and *R. japonica* (Sample 6).

	Rana sp. Sapporo 1	*Rana* sp. Obihiro 2	*R. ornativentris* 3	*R. dybowskii* Tsusima 4	*R. dybowskii* Ussuri 5	*R. japonica* 6
1	—	0.017	0.394	0.838	0.415	0.908
2	0.161	—	0.379	0.798	0.346	0.897
3	0.508	0.502	—	0.760	0.565	0.837
4	0.674	0.666	0.641	—	0.514	1.030
5	0.537	0.503	0.589	0.580	—	1.094
6	0.690	0.690	0.660	0.715	0.733	—

Distance–Wagner tree had low percentage of standard deviation (Fitch and Margoliash, 1967) of 4.918 (cophenetic correlation = 0.976). These measures indicate a very good overall fit of the tree to the original data.

In the first cladistic analysis using 95 alleles as characters and *R. japonica* as an outgroup, only one shortest tree, with a minimum of 119 steps and a consistency index of 0.739 was produced. The topology of this tree was not identical to that found in the Distance Wagner analyses; within the ingroup, the two samples of *R. dybowskii* formed a clade that was a sister group to the *R. ornativentris*— *Rana*. sp. clade.

The second analysis using 24 loci as characters and *R. japonica* as an outgroup also produced only one shortest tree with a minimum of 72 steps and a consistency index of 0.972. Again, the topology of this cladogram was different from the two phenograms or the cladogram produced by the independent allele model. In this tree, the two samples of *Rana*. sp. from Hokkaido formed a clade that is a sister group to the *R. ornativentris* and *R. dybowskii* assemblage.

Due to the different branching patterns produced by the different methods, the phylogenetic relationships among the samples remain ambiguous, but the consensus is a trichotomy of three lineages of brown frogs with 2n = 24 choromosomes. On the other hand, the results

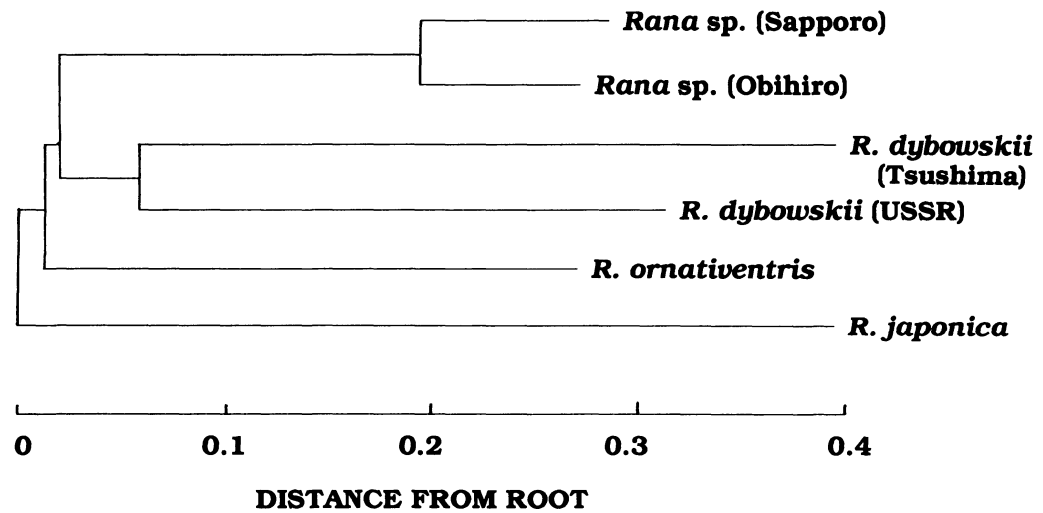

FIG. 3. Distance-Wagner tree constructed from modified Rogers' D (Wright, 1978) and rooted by the outgroup *R. japonica* for samples of *R.* sp. from Hokkaido, *R. ornativentris*, and *R. dybowskii*.

of the electrophoretic analyses clearly indicate that *Rana.* sp. from Hokkaido is genetically quite distinct from *R. ornativentris* or *R. dybowskii*.

On the basis of these morphometric and electrophoretic results, as well as hybridization results in the literature, I here describe *Rana* sp. from Hokkaido as:

Rana pirica sp. nov.
(Japanese name: Ezo-Aka-gaeru)
(Figs. 4–5)

Rana temporaria: Boulenger, 1886, p. 594 (part); Stejneger, 1907, p. 113; Okada and Kawano, 1923, p. 361.
R. temporaria chensinensis: Balcells, 1956, p.81; Okada, 1966, p. 74.
R. chensinensis: Kawamura, 1962, p. 185.
R. temporaria dybowskii: Nakamura and Uéno, 1963, p. 43.
R. chensinensis dybowskii: Nakamura and Uéno, 1965, p. 43.
R. sp.: Maeda and Matsui, 1989, p.74.

Holotype.—OMNH (Osaka Museum of Natural History) Am 9527, an adult male from Nakano-sawa, Minami-ku, Sapporo-shi, Hokkaido Prefecture, Japan (43°00' N, 141°19' E, 300m a.s.l.), collected on 9 October 1988 by Masafumi Matsui and Sen Takenaka.

Paratypes.—OMNH Am 9528, NSMT (National Science Museum, Tokyo)-H-04200-01, KUHE 9945, 9949–50, 9959, three males and four females, paratopotypes with the same collection data as the holotype; OMNH Am 9529–30, NSMT-H-04202-03, OCCM (Obihiro Centennial City Museum) AA 0111–12, KUHE 10169, 10171, six males and two females from Yachiyo-cho, Obihiro-shi, Hokkaido Prefecture (42°43' N, 142°57' E, 340m a.s.l.), on 21 April 1989 by Masafumi Matsui and Takanori Sato.

Referred specimens.—KUHE, unnumbered: two tadpoles, from Kushiro-shi, Hokkaido Prefecture, collected on 20 July 1978 by Teiji Sota.

Diagnosis.—A moderate-sized *Rana* (to about 72 mm SVL) of the *Rana temporaria* group; a dark mask covering tympanum; males with internal vocal sacs; diploid chromosome, 2n = 24; hindlimb short, tibiotarsal articulation of adpressed limb reaching at most anterior corner of eye.

Description of holotype.—Body robust; head short, slightly wider than long (Fig. 4A); snout triangular, but tip rounded in dorsal outline; projecting beyond lower jaw, slightly rounded in lateral profile; canthus rostralis fairly distinct; lores oblique, slightly concave; nostril below canthus, midway between tip of snout and anterior margin of upper eyelid; internarial distance greater than the distance from nares to eyes; eye moderate, horizontal diameter twice the eye-nostril distance and smaller than snout length; interorbital narrower than width of up-

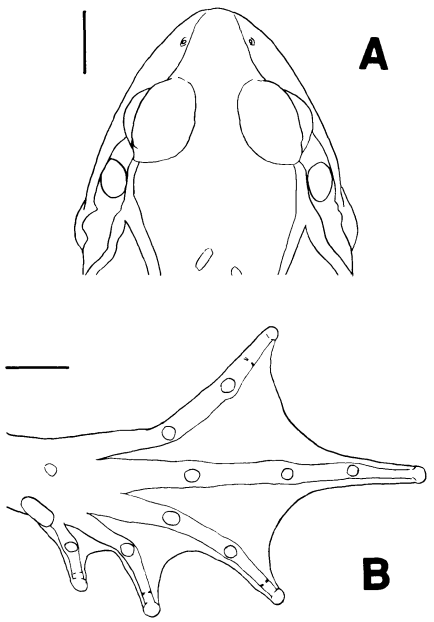

FIG. 4. Dorsl view of the head (A) and ventral view of the right foot (B) of holotype of *Rana pirica*. Scale indicates 5 mm.

per eyelid and internarial distance; tympanum distinct, nearly circular, about three–fifths eye diameter; vomerine teeth in oval, small, and slightly oblique raised series (each of 4 teeth), the center on line connecting posterior margins of choanae, narrowly separated from each other, but widely separated from choanae; tongue narrow anteriorly, moderately notched, without papilla; a pair of internal vocal sacs and vocal openings on corners of mouth.

Forelimb stout; fingers not slender, unwebbed; first finger only slightly longer than second, fourth longer than second; finger tips blunt, without disk; no fringes of skin along fingers; two large palmar tubercles, and a few, very weak supernumerary tubercles; subarticular tubercles prominent, round; distinct grayish nuptial pads on dorsal, medial, and ventral surfaces of the first finger extending from its base to a subarticular tubercle, covered with minute asperities that medially reach nearly to the finger tip.

Hindlimb short, about 2.6 times the length of forelimb; heels overlapping when limbs are held at right angles to body; tibiotarsal articulation of adpressed limb reaching anterior corner of eye; toe tips blunt, without disk; third toe subequal to fifth; toes moderately webbed (Fig. 4B), the following phalanges free of broad web: first toe, (1)—2nd toe, inner web (2), outer web (1)—3rd toe, inner web (2.5), outer web (1)—4th toe, inner web (2.5), outer web (2)—5th toe (1); excision of membrane between two outer toes reaching middle subarticular tubercles of fourth when toes are in contact; webs thick, not crenulate; subarticular tubercles prominent, round; inner metatarsal tubercle distinct, oblong, two–fifths length of first toe; outer metatarsal tubercle very feeble, round, one–third length of inner; sides of tarsus raised, but not forming distinct tarsal fold.

Dorsal skin scattered with small tubercles; a supratympanic fold from posterior margin of eye above and behind tympanum to above arm insertion; a dorsolateral fold from supratympanic fold to groin; sides scattered with small tubercles; throat and chest granular; abdomen coarsely granular, and rear half of thigh more densely so.

Color in alcohol (after formalin fixation).–Dark grayish brown dorsally on head and body; a narrow dark gray interorbital bar, a dark gray chevron marking in scapular region, and a pair of interrupted dark stripes on sacrum; no vertebral line; dorsolateral fold lighter internally, and with dark streak externally; lores with dark gray marking below canthus; a dark gray marking on whitish labial; a distinct dark gray marking from behind eye, covering tympanum and reaching above arm insertion; dorsal surfaces of limbs marked with alternating, dark crossbars; rear of thigh without marking; throat and abdomen whitish with indistinct dark markings scattered with guanophores, especially on throat; foot ventrally dark grayish.

Color in life.–Dorsum reddish brown; temporal mask and hindlimb bars dark grayish brown; throat, chest, and abdomen whitish, more yellowish postriorly covered with irregular grayish brown markings; ventral surface of legs bright yellowish orange; iris gold.

Measurements of holotype (in mm).–In preservative, method of measurement follows Matsui (1984), Matsui and Matsui (1990), and Maeda and Matsui (1989). SVL 54.1; HL (to rear of jaw commissure) 17.6; snout–tympanum rear edge length 16.7; jaw length 14.3; snout–nostril length 4.8; nostril–eyelid length 3.1; SL 7.9; EL 6.8; TD 3.4; HW 18.3; mouth width 16.3; IND 4.3; intercanthal distance 7.0; IOD 3.4; UEW 4.4; upper eyelid margin distance 12.6; forelimb length 34.8; LAL 26.2; third finger length 8.0; hand length 14.4; forearm width 5.9; HLL 91.4; THIGH 26.0; TL 27.6; FL 32.0; first toe length 6.7; fourth toe length 17.5; outermost toe web length 18.9; outer metatarsal tubercle length 1.0;

TABLE 4. Comparisons of measurements ($\bar{x} \pm 1$ SE, followed by ranges in parenthesis, in mm) in three species of Japanese brown frogs with the diploid chromosome number of 2n=24. See text for abbreviations.

Measurement	*Rana pirica*		*Rana ornativentris*		*Rana dybowskii*	
	Male N=15	Female N=11	Male N=10	Female N=10	Male N=9	Female N=7
SVL	54.94±0.72 (48.9−61.0)	64.35±1.37 (57.3−72.0)	50.87±1.72 (43.1−59.6)	65.76±2.41 (51.2−76.3)	56.53±1.22 (51.3−62.3)	67.55±1.98 (58.7−73.2)
HL	18.27±0.26 (16.4−20.3)	21.00±0.35 (19.2−22.8)	18.21±0.56 (15.9−21.0)	22.72±0.84 (18.5−27.2)	20.42±0.37 (18.6−22.3)	23.31±0.53 (21.1−25.2)
SL	8.17±0.13 (7.3−9.3)	8.77±0.17 (7.9−9.9)	7.79±0.25 (6.8−9.1)	9.65±0.36 (7.8−11.5)	8.33±0.15 (7.8−9.1)	9.83±0.21 (9.3−10.7)
EL	6.02±0.12 (5.2−6.7)	6.57±0.11 (6.2−7.2)	5.76±0.18 (4.7−6.6)	6.77±0.19 (6.0−7.8)	6.46±0.19 (5.8−7.3)	7.11±0.20 (6.2−7.8)
TD	3.48±0.13 (2.8−4.3)	3.85±0.14 (3.1−4.6)	3.64±0.14 (3.2−4.6)	4.42±0.13 (3.7−5.0)	3.88±0.13 (3.5−4.5)	4.34±0.20 (3.2−4.8)
HW	18.28±0.28 (16.4−20.2)	21.47±0.42 (19.8−24.3)	17.70±0.55 (17.9−21.5)	22.26±0.69 (18.9−25.7)	19.91±0.38 (15.4−21.0)	22.67±0.60 (20.7−25.3)
IND	4.47±0.09 (3.9−5.1)	4.99±0.07 (4.6−5.5)	3.83±0.10 (3.4−4.5)	4.49±0.14 (4.0−5.3)	4.51±0.14 (4.0−5.2)	4.77±0.10 (4.3−5.1)
IOD	3.31±0.07 (2.9−4.1)	3.73±0.15 (2.9−4.6)	2.89±0.09 (2.3−3.3)	3.58±0.16 (2.9−4.3)	3.45±0.13 (2.7−3.9)	4.02±0.10 (3.6−4.3)
UEW	4.75±0.12 (3.7−5.5)	5.37±0.12 (4.7−5.8)	4.32±0.15 (3.8−5.2)	5.43±0.19 (4.3−6.2)	4.70±0.16 (3.9−5.5)	5.57±0.11 (5.1−6.0)
LAL	26.67±0.44 (23.6−29.9)	28.44±0.41 (26.5−31.0)	24.75±0.73 (20.9−28.0)	29.51±1.13 (24.1−36.5)	27.53±0.31 (26.1−28.6)	30.18±0.66 (27.8−32.5)
HLL	91.29±1.49 (81.3−102.8)	98.07±1.83 (87.2−105.4)	93.39±3.90 (75.5−115.3)	117.32±5.12 (88.4−146.3)	106.0±1.90 (96.5−114.0)	120.35±3.53 (107.2−134.0)
THIGH	26.46±0.42 (23.8−29.0)	29.20±0.53 (26.7−32.2)	26.54±1.08 (22.0−35.3)	33.88±1.41 (26.0−42.0)	30.31±0.71 (27.5−33.4)	34.72±0.95 (31.0−37.1)
TL	27.45±0.39 (24.5−30.2)	29.41±0.52 (26.0−31.6)	28.98±1.19 (23.6−35.3)	37.04±1.56 (27.8−45.8)	32.56±0.60 (29.4−34.9)	37.55±0.95 (33.9−40.5)
FL	31.37±0.56 (27.8−35.8)	33.20±0.62 (29.8−36.1)	30.90±1.11 (25.6−36.4)	37.51±1.50 (29.3−45.3)	34.66±0.64 (30.7−36.8)	39.82±1.23 (34.8−45.5)
IMTL	3.15±0.07 (2.8−3.7)	3.60±0.14 (2.8−4.4)	3.04±0.11 (2.5−3.5)	3.60±0.10 (3.0−4.0)	3.18±0.11 (2.8−3.9)	3.70±0.12 (3.2−4.2)

IMTL 2.8.

Variation.–Morphometric data are summarized in Table 4 together with those on the allied species, *R. ornativentris* and *R. dybowskii*. Females are significantly larger ($\bar{x}$ = 64.3 mm) than males ($\bar{x}$ = 54.9 mm; t-test, $p < 0.01$). Relative lengths of lower arm, foot, and hindlimb are significantly greater in males (Mann–Whitney U-test, two-tailed, $p < 0.05$). Thus, when the hindlimb is bent forward along the body, tibiotarsal joint reaches between the anterior and posterior corners of eye in males, but in females, the joint at most reaches the posterior corner of eye (Table 5). Also, males have more developed toe webbing than females. In addition to ground body color metachrosis,

TABLE 5. Variation in the point reached by the tibio-tarsal joint when the hindlimb is bent forwards along the body. Figures indicate the number of specimens (percentage frequency in parenthesis).

	Tip of snout or forward	Beyond anter. edge of eye to snout	Beyond post. edge to anter. edge of eye	Post. edge of eye or behind
R. pirica				
Males	0	0	15 (100%)	0
Females	0	0	1 (9.1%)	10 (90.9%)
R. ornativentris				
Males	4 (40.0%)	6 (60.0%)	0	0
Females	2 (20.0%)	8 (80.0%)	0	0
R. dybowskii				
Males	9 (100%)	0	0	0
Females	2 (28.6%)	5 (71.4%)	0	0

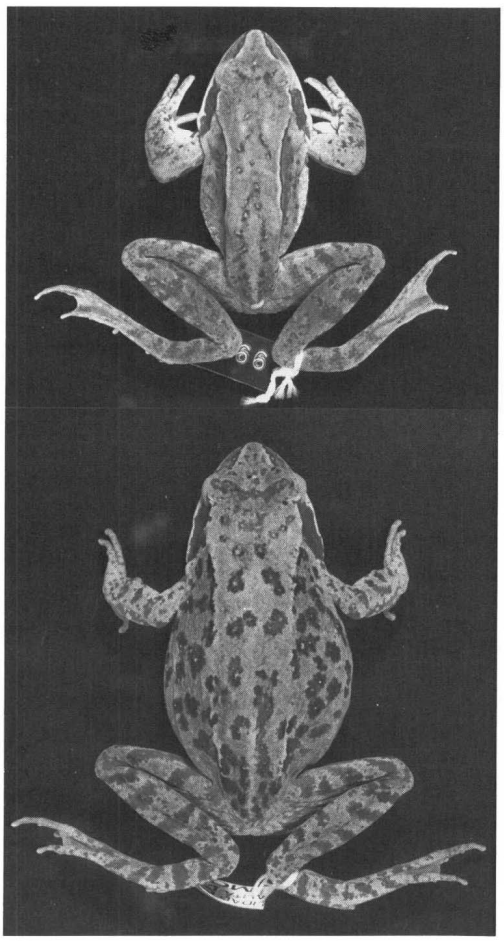

FIG. 5. Dorsl views of holotype (top) and female paratype (bottom : NSMT-H-04200) of *Rana pirica*.

metamorphosed individuals are fairly variable in coloration. The greatest variabilities occur in the extent of the dark mottling on the abdomen, and in the dark spots on the back (Fig. 5). Some specimens have tarsus more or less raised laterally to make the ventral side concave, but the others have tarsus without lateral elevation or ventral concavity.

Eggs and larvae.–Eggs are laid in a globular mass. The clutch size is 730–1,160. The diameter of 32 ova artificially squeezed from four females ranged from 1.7–2.3 ($\bar{x} \pm 2$ SE = 2.04 ± 0.07) mm. The animal pole is dark brown and the vegetal pole is grayish white in color. The swollen jelly layer is very tough.

For description of tadpoles, terminology follows Altig (1970). Oral disc small, weakly emarginate; lower jaw narrow; labial tooth rows 4(2–4)/4(1); A–2–4 gaps wide, A–2 gap ratio 1.5; P–1 gap narrow; without row of submarginal papillae between P–3 and marginal papillae; anus dextral; spiracle sinistral, opening constricted; eyes dorsal; tail moderately long and tip rounded; tail fin moderately high.

Measurements (in mm) of two tadpoles at stage 38 of Gosner (1960) are: total length 40.0 and 46.4, body length (BL) 14.8 and 15.2, body breadth 10.9 and 12.4 (74–82% BL), body height 8.2 and 11.0 (55–72% BL), interorbital distance 3.8 and 3.6 (24–26% BL), oral disc breadth 2.8 and 3.3 (19–22% BL), tail length 25.2 and 30.4 (170–200% BL), tail height 8.3 and 10.6 (56–70% BL), ventral fin height 59–78% of dorsal fin height, which in turn is 74–89% of musculature height.

General coloration (in alcohol) grayish brown dorsally, without a dark spot on each side, and grayish white ventrally with dense guanophores; tail paler than body, indistinctly blotched dark; gut only slightly visible.

SVL of juveniles at the time of metamorphosis ranges from 11–15 mm.

Karyotype.–2n = 24, with five large and seven small pairs. Chromosome Nos. 1, 3, and 5 in the larger group are metacentric, while Nos. 2 and 4 in the larger group and Nos. 6–10 and 12 in the smaller group are submetacentric. A small chromosome No. 11 is telocentric. The small chromosome No. 10 has a distinct secondary constriction in the longer arm (Seto, 1965, Nishioka et al., 1987).

Call.–Mating calls, recorded at Obihiro–shi, Hokkaido (water temperature = 6.5 C) were either single or consisted of call groups with 5–9 calls ($\bar{x}$ = 7.3, N = 3). Each call consisted of 3–11 ($\bar{x} \pm$ SE = 4.63 ± 0.40, N = 6) notes. The note duration varied from 86.2–119.8 ($\bar{x} \pm$ SE = 95.69 ± 1.27, N = 31) msec, and the mean call duration varied from 262 msec with 3 notes to 1056 msec with 11 notes (Table 6). In the call groups, inter–call duration varied from 196–394 ($\bar{x} \pm$ SE = 261.9 ± 15.8, N = 4) msec. Each note showed clear harmonics, each with conspicuous frequency modulation, and the dominant frequency measured at the highest point varied from 1278–1688 ($\bar{x} \pm$ SE = 1484.7 ± 21.1, N = 21) Hz, which corresponded to the second harmonic (Fig. 6).

Comparisons.–Several measurements for Chinese *R. chensinensis* from near the type locality (Qinling, Shaanxi) are given in Hu et al. (1966:60). These measurements are assumed to have been taken according to the method described by Liu and Hu (1961:4), and are not exactly

TABLE 6. Variation in the call duration (in msec) in the calls with different number of notes.

	N of notes					
	3	4	5	6	9	11
N	7	15	3	5	2	1
Range	262–359	351–404	431–496	520–633	842–887	—
$\bar{x}\pm SE$	300.4±12.4	372.5±3.6	479	591.1±19.9	864.5	1056

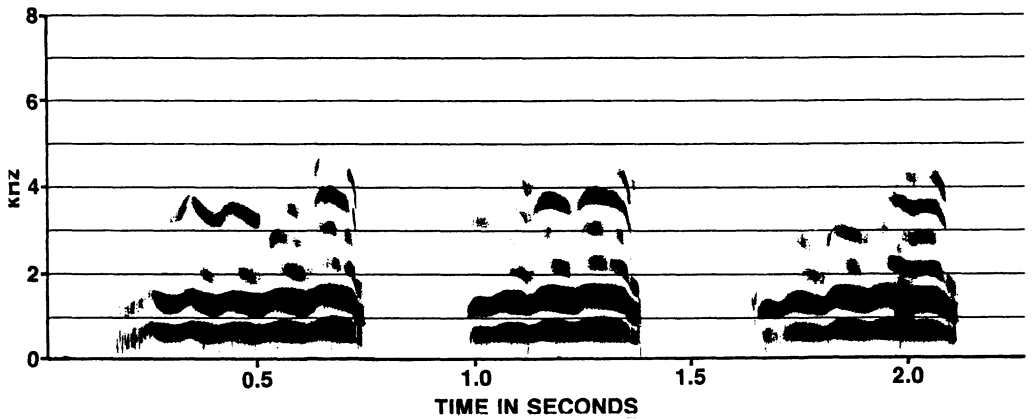

FIG. 6. Sonagram of mating call of *Rana pirica* from Obihiro-shi, Hokkaido, analyzed with wide band (300 hz) filter.

the same as those taken in the present study. There are, however, some morphological characteristics in which *R. pirica* assuredly differs from the topotypic population of *R. chensinensis* notwithstanding the possible difference in the manner of measurements; *Rana pirica* has a definitely larger body and shorter hindlimbs (mean SVL = 64.3 mm in females and 54.9 mm in males; mean HLL relative to SVL = 152.4 % in females and 166.2 % in males) than in *R. chensinensis* (mean body length = 48.4 mm in females and 46 mm in males; mean total hindlimb length relative to body length = 198.1 % in females and 184.5 % in males; Hu et al., 1966:61).

Among Japanese members of brown frogs, *R. pirica* most resembles *R. ornativentris* and *R. dybowskii*, both with 2n = 24 chromosomes, but differs from them in some morphological characteristics. *Rana pirica* has relatively short hindlimb (Table 4), and the tibiotarsal joint reaches at most to the anterior corner of the eye when the hindlimb is bent forward along the body even in males that have longer limbs than in females. Whereas in *R. ornativentris* and *R. dybowskii*, the joint at least reaches the point beyond the anterior corner of eye even in females (Table 5).

In *R. pirica*, the tympanum relative to eye is small and its longer diameter accounts for 45–72 % (median = 57 %) of the eye diameter, in contrast to 56–79 % (median = 63 %) in *R. ornativentris* or 50–72 % (median = 63 %) in *R. dybowskii*.

Rana pirica has a narrow interorbital space which is 73 % of the internarial and 67 % of the upper eyelid width, but in *R. ornativentris*, the interorbital is wider, accounting for 75 % of the internarial and 67 % of the upper eyelid. *Rana dybowskii* has an even wider interorbital, which is 79 % of internarial and 73 % of the upper eyelid.

In *R. pirica*, the vomerine teeth groups are small, and the centers usually lie on the line connecting the posterior borders of the choanae. The number of teeth on one side varies from 0 to 4 (medians = 2.5 in males and 3.5 in females). In *R. ornativentris*, the series are long, centers more frequently situated posterior to the "line". The number of teeth varies from 2 to 9 on each group (medians: male 5, female 6.5). *Rana dybowskii* has a large teeth series, centers usually posterior to the "line", and the teeth number is slightly larger, ranging from 3 to 4 (medians =

males 4.5, and females 5).

Rana pirica also differs from *R. ornativentris* and *R. dybowskii* in the following characteristics: thick dorsolateral fold; light stripe on middorsal line absent or only partially present; usually with dark markings covering the abdomen; outer metatarsal tubercle variously developed, but usually indistinct.

By contrast, *R. ornativentris* usually has a thin dorsolateral fold, light stripe on middorsal line, usually no dark markings on abdomen, and if present, not on the whole abdomen, and usually a distinct outer metatarsal tubercle. In *R. dybowskii*, the dorsolateral fold is usually thin, the light stripe on middorsal line is rarely present, dark markings on abdomen, if present, are limited, and the outer metatarsal tubercle is usually absent.

In addition, *R. pirica* differs from *R. ornativentris* in the following respects: canthus-rostralis is usually fairly distinct; always with a distinct dark streak below canthus from snout to eye; a chevron marking is usually absent, and indistinct if present; dark bars on hindlimbs are few in number, 2–4 (median = 3) on thigh and 2–5 (median = 3) on tibia. Whereas in *R. ornativentris*, canthus rostralis is usually indistinct, and often absent, a dark streak below canthus from snout to eye is indistinct, and often absent, a chevron marking is usually absent, and dark bars on the hindlimb are variable in number but are numerous, 2–7 (median = 4) on thigh and 3–6 (median = 4.5) on tibia.

From *R. dybowskii*, *R. pirica* also differs in having the following characteristics: dorsal skin with a few tubercles; dark bars on tibia slightly more numerous; usually with a few dark spots on flanks. Whereas in *R. dybowskii*, the dorsal skin is nearly smooth, dark bars on tibia are less numerous, 0–4 (median = 3) in number, and dark spots on flanks are frequently absent.

Range.–So far known only from Hokkaido, the northenmost large island of Japan, and adjacent small islands, Rishiri (Uéno, Personal communication) and Rebun Is. (Matsui, Unpublished data).

Natural history.–*Rana pirica* altitudinaly ranges very wide, from plains to montane regions up to 2,000 m in altitude. They breed in a short period during April and July in still waters in marshes, ponds and small pools. Eggs are laid in large masses, and each mass is tighted closely in shallows with a large portion of the egg mass above water. Embryonic and larval developments are swift, and usually the larvae do not hibernate even in high altitudes. Larvae are predated by a larval salamander, *Hynobius retardatus*.

Etymology.–The specific name is derived from "pirka", the language of the Ainu, native to Hokkaido Island, meaning beautiful or good, although the people themselves actually abominate this frog.

Discussion

Rana pirica is most likely a close relative of *R. dybowskii* and *R. ornativentris*, sharing with them the 2n = 24 chromosome number, but is both morphologically and genetically well differentiated from them. A Nei's D of 0.017, between the two samples of *R. pirica*, is regarded as a level of differentiation typical of populations within a species. On the other hand, *R. pirica* was separated from *R. ornativentris* by an average D of 0.387, and from the Tsushima and the Ussuri samples of *R. dybowskii* by an average D of 0.818 and 0.381, respectively. These values are judged to have reached a level that has been found to be typical among different species within the genus *Rana* (Avice and Aquadro, 1982), and therefore, *R. pirica* is judged to be genetically well differentiated from *R. dybowskii* and *R. ornativentris*, enough to be split at the specific rank.

Rana pirica is now known only from Hokkaido island, but fossil evidence indicates that the species was already in existent during the late Pleistocene (exact date unknown) in northernmost Honshu, together with *R. ornativentris* (Hasegawa et al., 1988). Hokkaido and Honshu are assumed to have been separated by the formation of the Tsugaru Strait during the Ris–Wurm interglacial period, approximately $10–15 \times 10^4$ years B. P. (Ohshima, 1990). Thus, the separation of *R. pirica* and *R. ornativentris* from their common ancestor is supposed have occurred well before 15×10^4 years B. P. *Rana ornativentris* was once reported to occur in Hokkaido (as *R. temporaria* var. *montana* by Okada and Kawano, 1923), but is now believed to be confined to the other large islands of Japan.

Rana pirica was long identified as the common European brown frog, *R. temporaria* (Boulenger, 1886; Stejneger, 1907; Okada, 1930, 1931; Okada and Kawano, 1923), but the latter species is now known to have 2n = 26 chromosomes and occur throughout Europe east to the Urals, except for most of Iberia, much of Italy, and the southern Balkans (Frost, 1985), not extending to the Asian regions. More recently, *R. pirica* is often regarded as a population of

R. chensinensis or *R. dybowskii* (Kawamura, 1962; Nakamura and Uéno, 1963, 1965; Frost, 1985), but *R. chensinensis* was originally described from Inkiapo, Lao-yu River, above 1,000 m in altitude in the Tsinling (=Qinling) Mountains, Shensi (= Shaanxi) Province, China by David (1875), which is quite distant from Hokkaido.

Within China, *R. chensinensis* is known from a wide range, from eastern Mongolia, through northeastern, central, and western China south to Sichuan and Hubei. It has long been pointed out that this species may include several distinct taxa (Liu and Hu, 1961; Hu et al., 1966). Hu et al. (1985:228) used a subspecific name *hongyuanensis* for the Hengduan population of *R. chensinensis*, and Wei and Chen (1990) proposed three subspecific names, *lanzhouensis*, *kangdingensis*, and *changbaishanensis*, within *R. chensinensis*. The nomenclatural validity of all these new names is doubtful, but the presence of distinct forms within *R. chensinensis* is quite certain.

The population of *R. chensinensis* from northeastern China is distinct from other populations in the characteristic swelling of water in the oviduct during preservation (Liu and Hu, 1961) as is *R. dybowskii* (Shannon, 1956) and *R. pirica* (as *R. chensinensis* from Hokkaido; Kawamura, 1962). This population is called the Hashima-frog, and the oviduct is economically important as a component of traditional Chinese medicine. From its distribution, the Hashima-frog seems to belong to a distinct subspecies of *R. chensinensis* proposed by Wei and Chen (1990), but may actually represent a good species, which has a sister relationship with *R. pirica* and *R. dybowskii*.

Rana dybowskii was described from Abrek Bay, near Vladivostok, USSR (Günther, 1876), and is found in the Far East USSR, Korean Peninsula, and Tsushima Island of Japan. From a biogeographic point of view, *R. pirica* would be considered closer in relationship to *R. dybowskii* than to *R. chensinensis*, and is apparently distinct from western Chinese *R. chensinensis*. Since artificial hybrids between *R. dybowskii* from Korea or Tsushima and *R. pirica* (as *R. chensinensis* from Hokkaido) are reported to be inviable or sterile (Kawamura et al., 1981), they evidently represent different species.

Within *R. dybowskii*, however, local populations seem to have genetically well differentiated. The present electrophoretic results indicate a remote genetic relationship between the Tsushima and Maritime populations of this species. The Nei's D of 0.514 obtained between the two samples is even larger than that found between the Maritime sample of *R. dybowskii* and *R. pirica* (D = 0.381), and relationships among the populations of brown frogs now collectively called *R. dybowskii* remain to be studied. Additional studies using continental samples are necessary to elucidate the phylogenetic relationships among relatives of *R. pirica*. Continental populations of a brown frog called *R. semiplicata* (Bannikov et al., 1977) seems to be synoymous with *R. dybowskii*, but the population of the same species from Sakhalin is possibly conspecific with the Hokkaido population, and a study of these brown frogs is now under way.

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Appendix I.

Specimens examined for morphometry.–The species and localities of samples used in the morphometric analyses are given below. The samples are deposited at the Graduate School of Human and Environmental Studies, Kyoto University (KUHE), Osaka Museum of Natural History (OMNH), National Science Museum, Tokyo (NSMT), and Obihiro Centennial City Museum (OCCM).

Rana pirica (N = 26, all from Hokkaido Pref.): Sapporo-shi, OMNH Am 9527, 9528, NSMT-H-04200, 04201, KUHE 9945, 9949–50, 9959; Obihiro-shi, OMNH Am 9529, 9530, NSMT-H-04203, OCCM AA 0111–12, KUHE 10165, 10168–71, 10179–80, 10183, 10186; Erimo-cho, KUHE 5454; Shimamaki-mura, KUHE 6204; Kamikawa-cho, KUHE 6276; Toyotomi-cho, KUHE 9942.

Rana ornativentris (N = 20): Shiobara-machi, Tochigi Pref., KUHE 6300–01; Okutama-machi, Tokyo Pref., KUHE 6318; Chosei-mura Chiba Pref., KUHE 10790, 10794; Ryotsu-shi, Niigata Pref., KUHE 10773, 10776, 10785–87; Oshimizu-machi, Ishikawa Pref., KUHE 10767–68; Kisofukushima-machi, Nagano Pref., KUHE 9004b; Miyama-cho, Kyoto Pref., KUHE 6273; Kyoto-shi, Kyoto Pref., KUHE 5525; Hiwa-cho, Hiroshima Pref., KUHE 6518; Kirishima-cho, Kagoshima Pref., KUHE 6202; Oguchi-shi, Kagoshima Pref., KUHE 5602, 5605–06.

Rana dybowskii (N = 17, all from Tsushima Is., Nagasaki Pref.): Kamiagata-cho, OMNH Am 2570, 2801–02, 2927–28; Mine-cho, TS (Sen Takenaka Collection) 33; Izuhara-machi, OMNH Am 5486, KUHE 11300; Mitsushima-cho, KUHE 11274–78, 11595–98.

Appendix II.

Specimens examined for electrophoresis.–The sample vouchers are deposited at the Graduate School of Human and Environmental Studies, Kyoto University (KUHE), Osaka Museum of Natural History (OMNH), and National Science Museum, Tokyo (NSMT).

Rana pirica (N = 19, all from Hokkaido Pref.): Sapporo-shi, NSMT-H-04201, KUHE 9945, 9949–52, 9957–58, 9963; Obihiro-shi, OMNH Am 9529, NSMT-H-04203, KUHE 10166, 10168, 10171–72, 10176, 10184–86.

Rana ornativentris (N = 9): Nikko-shi, Tochigi Pref., KUHE 10199, 10201; Itsukaichi-machi, Tokyo Pref., KUHE 11255; Okutama-machi, Tokyo Pref., KUHE unnumbered; Ryotsu-shi, Niigata Pref., KUHE 10771, 10778, 10785–86; Usuki-shi, Oita Pref., KUHE 8971.

Rana dybowskii (N = 10): Izuhara-machi, Tsushima Is., Nagasaki Pref., KUHE 11300; Mitsushima-cho, Tsushima Is., Nagasaki Pref., KUHE 11274–79; Lazo, Maritime, USSR, KUHE 11152–54.

Rana japonica (N = 8): Nikko-shi, Tochigi Pref., KUHE 10197; Otsu-shi, Shiga Pref., KUHE 9255–60; Minamiyamashiro-mura, Kyoto Pref. KUHE 10883.

要旨　エゾアカガエルの原記載

松井　正文

2n = 24 本の染色体をもつエゾアカガエル（北海道産）、ヤマアカガエル（本州、九州産）、チョウセンヤマアカガエル（対馬、ウスリー産）の系統分類学的関係を、形態学的・遺伝生化学的手法を用いて調査した。その結果、エゾアカガエルは形態的にも遺伝生化学的にも他の2種から明瞭に区別され、遺伝学的調査の結果を総合すると、3種のアカガエル類は共通祖先から3分岐したものと推定された。この結果と文献中にみられる交雑実験の結果とを考慮すると、エゾアカガエルは、ヤマアカガエル、チョウセンヤマアカガエルと別種として扱われるべきと判断される。さらに、しばしばエゾアカガエルと同一種とされる中国陝西省産のアカガエルは、文献資料からみる限り、形態的にエゾアカガエルと明瞭に区別され、分布の面からも両者が同一種である可能性は低いと考えられる。これらの事実に立脚して、北海道札幌産の雄個体に基づくエゾアカガエルを正式に記載し、新種名を与えた。本種は短い後肢をもつことで形態的に特徴づけられる。東アジア産で2n = 24本の染色体をもつアカガエル類の関係について論じ、チョウセンヤマアカガエルの対馬産とウスリー産との間で、遺伝的分化がかなり進行していることも示唆した。

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